SWAMI RAMANAND TEERTHMARATHWADA UNIVERSITY, NANDED

Synopsis

The research work leading to the degree of the

Doctor of philosophy

In

Zoology

Entitled

" Effects of Malathion Insecticide On Biochemical and Physiological Alterations of Freshwater Female Crab, *Barytelphusa guerini*".

By

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Head Dept. of Zoology

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Index

1) **Proposed Problem**
2) **Introduction**

a) Chemical Structure of Malathion

b) Degradation Pathway of Malathion

c) Physical Characteristics of Malathion

d) Effects on aquatic organisms

e) Breakdown in soil and groundwater

f) Breakdown in water

3) **Review of Literature:**
4) **Objective of Research:**
5) **Methodology:**
    a. Toxic Evaluation
    b. Behavioural Study
    c. Heart Beat Rate
    d. Oxygen Consumption
    e. Estimation of Protein
    f. Estimation of Glycogen
    g. Estimation of Lipid
    h. Estimation of Amino Acid
6) **Importance of Study**
    a. To Study Environmental Effects
    b. Effect on Crops and Soil and Life
    c. Biotic Poisoning
    d. Bio-Accumulation
7) **Proposed Work Plan with Chapter**
8) **Year wise Plan**
9) **Expected Outcome**
10) **References**

" Effects of Malathion Insecticide On Biochemical and Physiological Alterations of Freshwater Female Crab, Barytelphusa Guerini".

1) Proposed Problem:

Malathion is wide-spectrum organophosphate insecticide. It was one of the earliest organophosphate insecticides developed (introduced in 1950). Malathion is suited for the control of sucking and chewing insects on fruits and vegetables, and is also used to control mosquitoes, flies, household insects, animal parasites (ectoparasites), and head and body lice. Malathion may also be found in formulations with many other pesticides. However, Malathion is found to be highly toxic to various non-targeted aquatic organisms including Invertebrates and Vertebrates. Contributing factor to the sensitivity of fish to Malathion exposure seems to be its high rate of gill absorption due to the lipophilicity. The main mode of its action is neurotoxicity, and its capacity to induce oxidative stress or alteration of antioxidant system and lipid peroxidation.Thus,the main aim of this study is to review the toxic effect of Malathion in Aquatic Arthropods.

In recent years, the high rate of increase in human population and rapid pace of industrialization have created problem of disposal of waste waters. The domestic wastes and untreated or partially treated industrial effluents, supplemented with pollutants like heavy metals, pesticides and many organic compounds, have greatly contributed to massive fish death of aquatic ecosystems. These toxic chemicals and metals have changed the quality of water that affects the fish and other aquatic organisms. Pesticides are the major potential environmental hazards to humans and animals as these are present and concentrated in the food chain. The long-term ecological hazards associated with the use of organochlorine, organophosphate and carbamate pesticides has led to the introduction of a new generation of pesticides with a lesser degree of persistence.

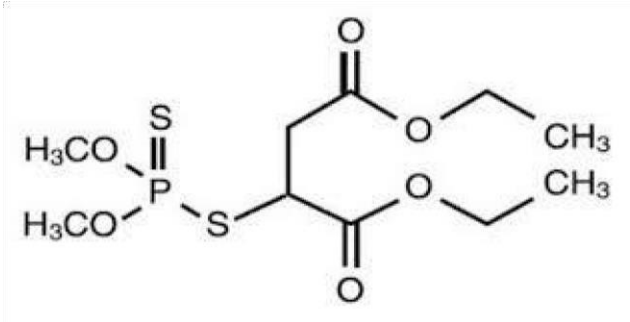
2) Introduction:

Malathion is an organophosphorus insecticide used in public health, residential, and agricultural settings as early as 1950. Over 100 food crops can be treated with Malathion, and about half of total applications in the United States (U.S.) are on alfalfa, cotton, rice, sorghum, and wheat. Annual use of Malathion is over 16.7 million per year of which approximately 12.5 million is used on food crops alone. The following uses constitute4.2 million per year with application to stored commodities, empty storage facilities, home and garden, golf courses, mosquito abatement, ornamental nursery stock and turf, Christmas tree plantations, parks, cemeteries, garbage areas, irrigation systems, outdoor dwellings (domestic and commercial), intermittently flooded areas, sewage systems, pastures, and rangeland (ATSDR, 2003). Malathion has been used in three major regional eradication programs in the U.S., specifically Boll Weevil eradication on cotton (11.2 million lbs/year), Medfly control (800,000 lbs/year), and mosquito control (472,000 lbs/year).A non-FIFRA pharmaceutical use of malathion is as a pediculicide for head lice and ova (U.S.EPA, 2000). In 2004, the Department of Pesticide Regulation (DPR) reported that 492,307 pounds of malathion were used in California (California Department of Pesticide Regulation's Pesticide Use Database. 2005).

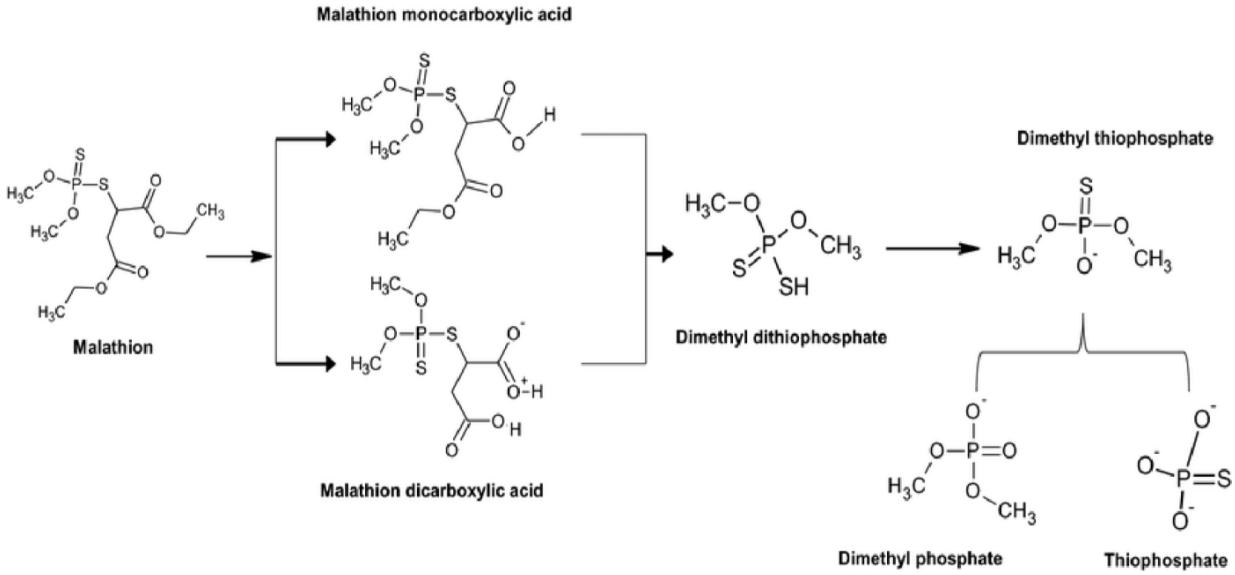
There are 38 basic producers of malathion and it is used in approximately 1,953 products worldwide (Meister Pro, 2003). Of those approximately 174 products are registered for use in the U.S. of which 36 products are currently registered for use in California (NPIRS, 2004). Malathion is formulated as an emulsifiable concentrate (EC), a dust (D), a wettable powder (WP), a pressurized liquid (PrL), and as ready-to-use liquids used for ultra-low-volume (ULV) application. Examples of common product names include Agrisect, Atrapa, Bonide, Prentox, Clean Crop malathion, Acme malathion, Black Leaf malathion spray, Eliminator, Fyfanon, and Gowan malathion dust.

Malaoxon is an oxygen analogue of malathion and it can be found either as an impurity in malathion product, or can be generated during the oxidation of malathion in air or soil. Malaoxon is the active cholinesterase inhibiting metabolite of malathion. Both malathion and malaoxon are detoxified by carboxyesterases to polar, water-soluble compounds that are excreted.

Chemical Structure of Malathion



Degradation Pathway of Malathion



Physical Characteristics of Malathion

Composition: Diethyl(dimethoxy thiophosphorylthio) succinate Properties : Clear to amber liquid with a mercaptanodor. -93

- Henry's Law Constant:4.89X10atm/m mol
- Specific Gravity:1.23at25°C
- Melting Point: 2.85°C
- Molecular Weight:330.36
- Water Solubility:130mg/L
- Partition Coefficient:2.7482 □AdsorptionCoefficient:1,800-6
- Vapor Pressure:3.38X10 mmHg at 30°C
- Miscible in most organic solvents ; limited solubility in aliphatic hydrocarbon.

Effects on aquatic organisms:

Malathion has a wide range of toxicities in fish, extending from very highly toxic in the walleye (96-hour LC50 of 0.06 mg/L) to highly toxic in brown trout (0.1 mg/L) and slightly toxic in goldfish (10.7 mg/L). Malathion is highly toxic to aquatic invertebrates and to the aquatic stages of amphibians. Because of its very short half life, malathion is not expected to bio concentrate in aquatic organisms. However ,brown shrimp showed an average concentration of 869 and 959 times the ambient water concentration in two separate samples.

Effects on other organisms :

The compound is highly toxic to honeybees.

Breakdown in soil and groundwater:

Malathion is of low persistence in soil with reported field half-lives of 1to25days.Degradation in soil is rapid and related to the degree of soil binding. Breakdown occurs by a combination of biological degradation and non biological reaction with water. If released to the atmosphere, malathion will break down rapidly in sunlight, with a reported half-life in air of about 1.5 days. It is moderately bound to soils, and is soluble in water, so it may pose a risk of groundwater or surface water contamination in situations which may be less conducive to breakdown.

Breakdown in water:

In raw river water, the half-life is less than 1 week, whereas malathion remained stable in distilled water for 3 weeks. Applied at 1 to 6 lb/acre in log ponds for mosquito control, it was effective for2.5to6weeks.In sterile sea water, the degradation increases with increased salinity. The breakdown products in water are mono- and dicarboxylic acids.

3) Review of Literature:

1. Kadiru, S., Patil, S., & D'Souza, R. (2022). Effect of pesticide toxicity in aquatic environments: A recent review. International Journal of Fisheries and Aquatic Studies, 10(3), 113-118.

Analysis: This comprehensive review establishes the foundation for understanding organophosphate pesticide contamination in Indian aquatic ecosystems. Despite lower per-hectare pesticide usecompared to developed countries, injudicious application has resulted in widespread residues in major river systems (Ganges, Yamuna, Cauvery, Tapi). Agricultural runoff serves as the primary contamination vector, with bioaccumulation through food chains posing risks to non-target aquatic species including freshwater crabs.

2. de Oliveira, C., Sabóia-Morais, S. M., Lombardi, C. A., Freire, C. A., & Neves, M. C. (2019). Neurotoxicity of organophosphate pesticides could reduce the ability of fish to escape predation

under low doses of exposure. Scientific Reports, 9, 11614.

Analysis: Demonstrates that sub lethal organophosphate exposure (ethoprophos at 0.01 mg/L) causes 53.6% brain cholinesterase inhibition, correlating with impaired escape responses. This neuro toxic effect at concentrations 50 times lower than LC50 highlights the extreme sensitivity of aquatic organisms and establishes behavioral modification as a primary sublethal endpoint relevant to behavioral observation methodology.

3. Costa, L. G. (2018). Organophosphorus compounds at 80: Some old and new issues. Toxicological Sciences, 162(1), 24-35.

Analysis: Provides authoritative overview of organophosphate mechanisms, emphasizing differential toxicity due to metabolic activation (malaoxon formation) and detoxification efficiency via carboxyesterases and glutathione-S-transferases. Explains species-specific sensitivity variations critical for understanding why freshwater crabs exhibit particular vulnerability to malathion exposure.

4. Hodgson, E., Philpot, R. M., & Kayama, A. K. (1993). Species differences in the metabolism of xenobiotics. In: Introduction to Biochemical Toxicology (pp. 55-78). Appleton and Lange.

Analysis: Classic reference establishing malathion's oxidative conversion to more toxic malaoxon metabolite.Documentsdetoxificationpathwaysconvertingbothcompoundstowater-solubleexcretable forms. Critical for understanding why malathion exhibits variable toxicity across aquatic species based on carboxylesterase efficiency.

5. Cohen, S. D., & Murphy, S. D. (1971). Metabolism of organic phosphorus pesticides. In: Insecticide Biochemistry and Physiology (pp. 249-313). Plenum Press. 5

Analysis: Foundational study documenting organophosphate-induced reactive oxygen species (ROS) generation and subsequent antioxidant defense activation. Establishes lipid per oxidation as primary cellular damage mechanism, providing theoretical basis for biochemical assays measuring protein, glycogen, and lipid alterations.

6. Barata, C., Lemos, M. F., Sousa, A., & Rios, G. (2004). Evaluation of cholinesterase activity asa biomarker of organophosphate pesticide exposure. Aquatic Toxicology, 68(3), 273-280. 6

Analysis: Establishes >70%AChE inhibition threshold for mortality prediction in aquatic invertebrates. Demonstrates cholinesterase monitoring enables rapid exposure assessment and population-level effect prediction, validating enzymatic biomarkers for research design.

7. Pathiratne, A., & George, K. C. (1998). CYP3A protein induction in tilapia (Oreochromis niloticus) following environmental xenobiotic exposure. Aquatic Toxicology, 42(2), 101-115.

Analysis: Documents 100% increase in CYP3A detoxification enzymes following organophosphate exposure, establishing enzyme inductions primary defense mechanism. Provides comparative baseline for expected detoxification responses in malathion-exposed crabs.

8. Pugazhvendan, S. R., Narendiran, J. N., Kumaran, R. G., Kumaran, S., & Alagappan, K. M. (2009). Effect of Malathion Toxicity in the Freshwater Fish Ophiocephalus punctatus - A Histological and Histochemical Study. World Journal of Fish and Marine Sciences, 1(3), 218-224.

Analysis: Sub lethal malathion (10-20 µl/L) exposure caused profound behavioral and respiratory alterations with histological protein fraction changes. Establishes behavioral screening and respiratory depression as reliable toxicity indicators consistent with methodology.

9. Thenmozhi, C., Vignesh, V., Thirumurugan, R., & Arun, S. (2011). Impacts of Malathion on mortality and biochemical changes of freshwater fish Labeo rohita. Iranian Journal of Environmental Health Sciences and Engineering, 8(4), 387-394.9

Analysis: Acute malathion exposure produced dose-dependent decreases in nucleic acids, proteins, free amino acids, and glycogen with partial recovery post-exposure. Directly supports biochemical parameter measurements and establishes metabolic disruption patterns.

10. Abd El-Gany,M. A., Zayed, A. A., & Ismail, A. A. (2022). Impact of malathion toxicity on the oxidative stress parameters of Hermetia illucens larvae. Scientific Reports, 12, 4896.

Analysis: Demonstrates 3.11-fold lipid per oxidation increase at 0.015 mg/ml malathion with compensatory SOD/CAT elevation. Establishes oxidative stress biomarkers as sensitive indicators of malathion toxicity relevant to lipid and protein assays.

11. Patil, V. N., Kadam, G. H., & Raut, P. D. (2014). Enzymological alterations produced by chronic malathion exposure in freshwater crab, Paratelphusa (Barytelphusa) jacquemontii. International Journal of Science, Environment and Technology, 3(1), 303-313. 11

Analysis: 30-day sub lethal malathion (0.555 p pm) exposure in co generic crab species produced significant metabolic enzyme alterations. Directly relevant species comparison establishing enzyme activity changes as reliable stress biomarkers.

4) Objective of Research:

Malathion have been the most commonly used insecticides since the early 1950s. Despite their efficacy in improving crop protection and management, these agrochemicals have gained recent attention for their negative impacts on non-target species such as honey bees and aquatic invertebrates.

In recent years, Malathion have been detected in rivers and streams across the world. Determining and predicting the exposure potential of Malathion in surface water requires a thorough understanding of their fate and transport mechanisms. Therefore, our objective was to provide a comprehensive review of Malathion with a focus on their fate and transport mechanisms to and within surface waters and their occurrence in waterways throughout the world. A better understanding of fate and transport mechanisms will enable researchers to accurately predict occurrence and persistence of insecticides entering surface water sand potential exposure to non-target organisms in agricultural intensive regions. This review has direct implications on how Malathion are monitored and degraded in aquatic ecosystems. Further, an improved understanding of the fate and transport of Neonicotinoid aide natural resource practitioners in the development and implementation of effective best management practices to reduce the potential impact and exposure of Malathion in waterways and aquatic ecosystems.

1. To determine the acute toxicity (96-hour LC50 and sub-lethal concentrations) of the organophosphateinsecticideMalathiononthefreshwaterfemalecrab,Barytelphusa guerini using the probit analysis method.

2. To investigate the behavioral alterations induced by acute and sub-lethal exposure to Malathion in Barytelphusa guerini as a rapid bio indicator of environmental stress.

3. To evaluate the physiological effects of Malathion on key parameters such as heartbeat rate and oxygen consumption rate in female Barytelphusa guerini.

4. To assess the impact of sub-lethal concentrations of Malathion on major biochemical constituents(totalprotein,glycogen,totallipids,andfreeaminoacids)inthehepatopancreas, muscle, and gill tissues of female Barytelphusa guerini.

5. To study the pattern of metabolic disturbance and possible recovery (ifany) in biochemical and physiological parameters in Barytelphusa guerini after exposure to Malathion and subsequent transfer to toxicant-free water.

6. To correlate the observed biochemical and physiological alterations with the known mode of action of Malathion (acetylcholine nest erase inhibition, oxidative stress, and energy metabolism disruption) in aquatic invertebrates.

7. To provide scientific data on the sensitivity of the fresh water crab Barytelphusa guerini as a non-target aquatic organism to Malathion contamination, contributing to ecological risk assessment of organophosphate insecticides in freshwater ecosystems.

5) Methodology:

1) **Toxic Evaluation :** LC_{50} value method used Followed By Finney(1964)
2) **Behavioural Study:** Quick Screening procedure for monitoring effects of Environmental Pollution (Janardhan Reddy 1988)
3) **Heart Beat Rate:** By Crab Ringer Solution.
4) **Oxygen Consumption:** Wrinkler Idometric Method(WelshandSmith,1953)
5) **Estimation of Protein:** Lowry's Method(Lowry,OH,1951)
6) **Estimation of Glycogen:** Anthrone Reagent Method(Selfers, etal,1956)
7) **Estimation of Lipid:** Methanol: Chloroform Method(Floch, J.etal., 1956)
8) **Estimation of Amino Acid:** Ninhydrin Method(RosenH.,1957)

6) Importance of Study:

1) **To Study Environmental Effects**

   Chemical pesticides are responsible for polluting our environment while being used in the field. Although their effects are short lived, they may begin causing hazardous environmental and health problems if they persist in the environment. When chemical pesticides are sprayed on the crops, they may spread to regions beyond the intended area.

2) **Effect on Crops and Soil and Life**

   Pesticides degrade the quality of soil and further the quality of the food and yield. Chemical pesticides deplete the nutritional value of the food and contaminate it. When we eat any food such as Vegetables and fruits like apple, lettuce, potatoes or strawberries, these pesticides enter our body and may lead to many health issues. While these chemical pesticides are developed to kill organisms found on the crop, these are also harmful to other living things.

3) **Biotic Poisoning:**

   Pesticides have also been shown to disrupt the balance of an ecosystem. In many situations, when a pesticide is used, it also kills non-pest organisms. This can drastically alter the natural balance of the ecosystem. By removing non-pest organisms, the environment can be changed to favor the pest. In addition to causing harm to wildlife, pesticides that travel from

their original location are known to cause harm to humans. Human exposure to pesticides has caused poisonings, the development of cancer and the deaths of between 20,000 and 40,000 people worldwide each year.

4) Bio Accumulation:

Another major problem associated with pesticide use is bioaccumulation and biological magnification. Bioaccumulation is when a substance builds up in the body because the body does not have the proper mechanisms to remove it. Many synthetic pesticides are not able to be broken down. Once they enter the body of an organism, they are permanently stored in the body tissue.

7) Proposed Work Plan with Chapter:

1) Chapter I : Introduction

The great solvent power of water has been making the creation of absolutely pure water a theoretical rather than a practical Goal. Water contamination by pesticides and Insecticides is serious problem. The awareness and option is needing full to protect the Nature and Balancing the Ecological equilibrium. All Collective Current and Previous information collected in this Chapter.

2) Chapter-II: Material and Method:

TheeffectofMalathion,InsecticidecausesvariouschangesinMetabolicaswellas Physiological activity in aquatic invertebrate female crab Barytelphusa guerini.

Acute toxicity accelerates deposition and make residual problem in tissues they make rushes in protein, Carbohydrates, Lipid, Amino acid and Nucleic Acid.

This Chapter Follows the Scientific protocols

1) **Toxic Evaluation** : LC50valuemethodused Followed by Finney(1964)

2) **Behavioural Study**: Quick Screening procedure form on storing effects of Environmental Pollution (Janardhan Reddy 1988)

3) **Heart Beat Rate:** By Crab Ringer Solution.

4) **Oxygen Consumption**: Wrinkler Idometric Method(WelshandSmith,1953)

5) **Estimation of Protein**: Lowry's Method(Lowry,OH,1951)

6) **Estimation of Glycogen** : Anthrone Reagent Method(Selfers, etal,1956)

7) **Estimation of Lipid**: Methanol: Chloroform Method(Floch,J.etal.,1956)

8) **Estimation of Amino Acid**: Ninhydrin Method(RosenH.,1957)

3) Chapter-III: Toxicity Evaluation:

The contamination of environmental pollution is becoming serious problem because of perfuse use of pesticides. Chemicals in agriculture, Industry and Heavy metals.

The poison is worst and requires safety measures in the place where industrial discharge carries the toxicant in proportion. The toxic evaluation of Malathion to the fresh water Female crab *Barytelphusa guerini* was detected precise dosages level, LC_{50} value method.

4) Chapter-IV: Behavioural Study:

The Behaviour represent an integrated response of the organism in the functioning of any one of the body systems results in behavioural change. It would form one of the quick screening procedures for monitoring the effect of environmental pollution view of this it would be appropriate to review certain behavioural abnormalities in crab treated with Malathion.

5) Chapter-V:Result and Discussion:

The acute toxic effect on various metabolite variations might be in all possibility due to differenceinthebiochemicalconstructionofthetissuesandBloodinrespecttotoxicant.Inthis chapter effect of Malathion, titled observation and tabulates by various tools of Statistical method. In this added all observations and results with tables and Graphs in relation to topic sited as

1) Heart Beat Rate: By Crab Ringer Solution.

2) Oxygen Consumption: Wrinkler Idometric Method(Welsh and Smith, 1953)

3) Estimation of Protein : Lowry's Method(Lowry,OH,1951)

4) Estimation of Glycogen: An throne Reagent Method(Selfers,etal,1956)

5) Estimation of Lipid: Methanol: Chloroform Method (Floch, J. et al., 1956) 6) Estimation of Amino Acid : Ninhydrin Method (Rosen H., 1957)

6) Chapter: VI: Summary and Conclusion:

The result and observations of the research work is summarized in summary and Conclusion.

7) Chapter-VII: Bibliography:

References used and site din literature are listed in the Bibliography.

8) Year wise Plan:

Year 1 Plan

Months 1-6 (Literature and Acute Toxicity)

Compile comprehensive literature for Chapter I (Introduction), covering Malathion's toxicity, ecological risks, and prior studies on aquatic invertebrates. Determine 96-hour LC50 and sub-lethal concentrations for Malathion on female Barytelphusa guerini using probit analysis (Finney 1964). Draft initial sections of Chapter II (Materials and Methods) with toxicity protocols and crab maintenance details. 1

Months 7-12 (Behavioral Studies)

Investigate behavioral alterations (e.g., locomotion, avoidance, responsiveness) under acute and sub-lethal Malathion exposures as bioindicators (Janardhan Reddy 1988). Analyze data for dose-response patterns to support Chapter III (Toxicity Evaluation) and Chapter IV (Behavioural Study). Begin statistical validation of LC50 and behavioral metrics.

Year 2 Plan

Months 13-18 (Physiological Evaluations)

Evaluate physiological effects: measure heart beat rate (Crab Ringer Solution) and oxygen consumption (Winkler Idometric Method, Welsh and Smith 1953) in exposed crabs. Correlate changes with exposure levels for Objective 3. Integrate findings into Chapter V (Results and Discussion) with preliminary graphs/tables. 1

Months 19-24 (Biochemical Assessments)

Assess impacts on biochemical constituents (total protein via Lowry's Method 1951; glycogen via Anthrone Reagent, Selfers et al. 1956) in hepato pancreas, muscle, and gill tissues under sub-lethal exposures (Objective 4). Initiate studies on metabolic disturbances and recovery patterns post-transfer to clean water (Objective 5).

Year 3 Plan

Months 25-30 (Advanced Biochemistry and Recovery)

Complete biochemical analysis : to tallipids (Methanol-Chloroform, Flochet al. 1956) and free amino acids (Ninhydrin, Rosen 1957) across tissues. Fully document recovery trends in biochemical/physiological parameters after Malathion depuration (Objectives 4-5). Perform correlations with Malathion's mechanisms (AChe inhibition, oxidative stress; Objective 6).

Months 31-36 (Synthesis and Risk Assessment)

Analyze all data statistically to highlight B. guerini's sensitivity as a non- target species (Objective 7). Draft Chapter VI (Summary and Conclusion) with ecological risk insights and Chapter VII (Bibliography). Revise the thesis, prepare visuals, and outline implications for freshwater pesticide management.

9) Expected Outcome

There search work having following outcomes:

1) Malathion is high deposition potential causes degrade molecular building in invertebrates, We got deposition ratio.
2) It causes decline rate of Metabolism like Respiration, effect of Heartbeat rate etc.
3) It shows effect on Natural source of Protein.
4) Residual problem cause cancer possibility to human kind as circulating contaminated food.

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